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EDITORIAL

Dialysis fluids and monocytes: Suicide or murder?

Continuous ambulatory peritoneal dialysis is a common procedure that frees some patients from constant dependence on hemodialysis centers. It is not without risk, especially that of infection, which can be catastrophic. Does the procedure itself impair local scavenger and immune function? After all, when one places a foreign solution into the peritoneum, the cells in contact with it may have their function or their very survival compromised. It is this question which Joerg Plum and colleagues address in this issue of *Kidney International* [1]. There are a number of ways in which the effects of peritoneal dialysis solutions on cellular function might be investigated, and here peripheral blood monocytes were chosen as a stand-in for resident peritoneal macrophages. This is reasonable, since it would be impossible to harvest human peritoneal cells with each of the test solutions, which are not approved in their tested form for *in vivo* use. Instead, fresh monocytes were incubated in a variety of solutions for 15 minutes, and then diluted with the most popular tissue culture medium, RPMI 1640, supplemented with fetal calf serum, which provides multiple growth factors. A number of tests were performed on the cells after a further 16 hours of incubation at 37°C. The authors feel that this is a reasonable model because, as they point out, the fluid in the peritoneum will equilibrate towards the composition of normal tissue fluid (with respect to pH and osmolality, at least) in about this time frame. Although not a perfect representation of what must go on in the body, it seems to me that this model would be useful at least for identifying solutions that are particularly good or bad at maintaining monocyte function. The solutions tested differed in their buffer (bicarbonate, phosphate, or lactate) and their energy source/osmolite (glucose, dextrin, or amino acids), as well as pH (lactate-buffered solutions were tested at both pH 5.5 and 7.5). Not every combination was tested, nor were mixed solutions, for example, ones containing both glucose and amino acids.

Further tests with a more systematic range of compositions are still needed. In all cases, comparisons were made to cells incubated 16 hours in RPMI 1640; values for this control were set to 100%. This makes the assumption that RPMI is the perfect medium, maintaining cells for 16 hours in peak condition, but it would have been useful to know what all values were when the cells were fresh. Viability was determined by a test of mitochondrial function, which showed that the lactate buffered solutions were least able to maintain viability, while glucose-bicarbonate and amino acid-bicarbonate were the best. This pattern repeated in a number of functional studies.

Perhaps the most interesting is the examination of the mode of monocyte death. Hematopoietic cells, like most others in the body, die by necrosis or apoptosis [2]. Necrosis is a violent sort of death, which follows abrupt, irreparable injury. It is sometimes likened to murder. Apoptosis, on the other hand, is suicide; it is a physiological process that cells can do to themselves when so signaled by other cells, by a change in their environment, when they reach a certain age, or when they are more mildly injured. Why would an injured cell want to commit suicide? Because its injury, if inaccurately repaired, might lock in a dangerous mutation. Lymphocytes are very ready to undergo apoptosis on the slightest injury, because if they go wrong, their propensity for rapid division represents a very significant threat to the body. Monocytes are less threatening, and as nicely demonstrated in the paper by Plum et al, they do not undergo apoptosis to any interesting extent under the experimental conditions (no solution induced more apoptosis than the "background" level of 20% seen with RPMI 1640). However, the lactate buffered solutions produced excessive death by necrosis. This is perhaps not surprising, as the concentration of lactate in the test solutions is about four times that achieved in the blood of healthy people at maximal exertion [3]. The method used to distinguish necrosis from apoptosis is worthy of examination, as it is very elegant indeed. In apoptosis, well before cells die the membrane becomes rearranged, and one of the phospholipids, phosphatidylserine, moves to the outer layer from its normal confinement within the cell [4]. Thus exposed, it can be detected with fluorescently-labeled annexin V [5]. Cells were also treated with propidium iodide, which bound to the DNA double helix, becoming fluorescent, but it could only penetrate cells whose membrane had become permeable in death. The flow cytometer distinguished annexin and propidium fluorescence, and displayed each on a separate axis (Fig. 2 of [1]). With RPMI 1640, most cells were negative for both stains, that is, normal. Some were annexin positive but propidium negative (sector III), that is, they were apoptotic but not yet dead. Necrotic cells were both permeable (propidium positive) and their membrane was disrupted (annexin positive) so they displayed in sector I. In the most toxic glucose-lactate condition, the flow

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cytometer diagram showed a dramatic sweep of cells into this sector.

RPMI 1640 was developed in Buffalo, New York, at Roswell Park Memorial Institute (hence the acronym) by George Moore [6]. It has become the medium of choice for both mouse and human hematopoietic cells. Since it was clearly best at preserving cell viability and function of all tested solutions, one wonders why it is not used for peritoneal dialysis. The main reason must be cost; it is a very complex mixture (at least 40 ingredients), and a liter sells for $16 US at wholesale. If one examines the contents [7] it seems to be an average of the solutions tested. That is, it contains glucose, amino acids, and bicarbonate; furthermore, its osmolality is close to physiological. Given the results presented in the interesting study by Plum et al, it seems clear which direction further research in peritoneal dialysis fluids should take.

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